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Washington, D.C. 20231

STATEMENT UNDER 37 CFR §1.55(a)

In accordance with 37 CFR §1.55(a), I hereby state that the content of the attached English translation of the certified copy of Japanese Patent Application No. 341604/1998, from which the present application claims priority, is accurate.

Respectfully submitted,

Date: This 6th day of November, 2000

T. Watanabe
Toshiaki Watanabe
Applicants' Japanese Representative

I hereby certify under 37 CFR §1.8(a) that this correspondence is being deposited with the United States Postal Service as first class mail with sufficient postage on the date indicated below and is addressed to the Assistant Commissioner for Patents, Washington, D.C. 20231.

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Deborah Dean

(Translation)

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This is to certify that the annexed is a true copy of the following application as filed with this Office.

Date of Application: December 1, 1998

Application Number: Japanese Patent Application No. 341604/1998

Applicant(s): Hitachi Software Engineering Co., Ltd.

November 19, 1999

Commissioner,
Patent Office Takahiko Kondo (seal)

Certificate No. 11-3079039

[Name of document] Patent Application

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[Title of the Invention] BIOCHIP AND METHOD FOR PRODUCING THE SAME

[Number of claims] 7

[Inventor]

[Address or Residence] c/o Hitachi Software Engineering Co., Ltd., 6-81, Onoe-cho, Naka-ku, Yokohama-shi, Kanagawa, Japan

[Name] Toshiaki ITO

[Address or Residence] c/o Hitachi Software Engineering Co., Ltd., 6-81, Onoe-cho, Naka-ku, Yokohama-shi, Kanagawa, Japan

[Name] Kenji YAMAMOTO

[Address or Residence] c/o Hitachi Software Engineering Co., Ltd., 6-81, Onoe-cho, Naka-ku, Yokohama-shi, Kanagawa, Japan

[Name] Toshimasa WATANABE

[Address or Residence] c/o Hitachi Software Engineering Co., Ltd., 6-81, Onoe-cho, Naka-ku, Yokohama-shi, Kanagawa, Japan

[Name] Noriko YURINO

[Applicant]

[Identification Number] 000233055

[Name] Hitachi Software Engineering Co., Ltd.

[Agent]

[Identification Number] 100091096

[Patent Attorney]

[Name] Yusuke HIRAKI

[Agent]

[Identification Number] 100102576

[Patent Attorney]

[Name] Toshiaki WATANABE

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[List of Attached Documents]

[Name of Document] Specification 1

[Name of Document] Drawing 1

[Name of Document] Abstract 1

[General Power of Attorney No.] 9722155

[Necessity of Proof] Necessary

[Document Name] Specification

[Title of the Invention] BIOCHIP AND METHOD FOR PRODUCING THE SAME

[Claim]

[Claim 1] A biochip comprising probes spotted on a plate at a plurality of positions by using a binding agent for binding the probes to the plate, wherein the binding agent is locally spotted at positions where the probes are spotted.

[Claim 2] A method for producing a biochip by spotting probes on a plate by using a binding agent for binding the probes to the plate, the method comprising a step of spotting mixtures of respective probes and the binding agent on the plate.

[Claim 3] A method for producing a biochip by spotting probes on a plate, the method comprising the steps of:

spotting a binding agent for binding the probes to the plate at positions where the probes are to be spotted; and

spotting the probes on the plate at positions where the binding agent is spotted.

[Claim 4] The method for producing a biochip according to claim 2 or 3, wherein the plate is made of glass.

[Claim 5] The method for producing a biochip according to any of claims 2 to 4, wherein the probes are spotted by using a pin with a recessed tip.

[Claim 6] A pin used for spotting a probe on a plate, wherein a tip of the pin is recessed.

[Claim 7] A pin used for spotting a probe on a place wherein a tip of the pin is provided with a cross shape groove.

[Detailed Description of the Invention]

[0001]

[Field of the Invention]

The present invention relates to a biochip comprising a place

spotted with various probes.

[0002]

[Prior Art]

Conventionally, biochips are produced by spotting various biopolymer probes such as DNAs, RNAs and proteins on a plate (e.g., a glass plate). Figure 4 is diagrams for illustrating the principle of such conventional technique. As shown in Figure 4(a), a microplate 2 containing various probe DNAs 1 is prepared. On the other hand, as shown in Figure 4(b), a glass place is prepared as a plate 3. As shown in Figure 4(c), the surface of the plate 3 is coated with poly-1-lysine as a binding agent 4 for binding the DNAs 1 to the glass. Thereafter, as shown in Figure 4(d), probe DNAs 1 in the microplate 2 are transferred by a pin 5 and spotted onto the glass plate 3 coated with the binding agent 4 for binding DNA and glass (poly-1-lysine). This process is repeated for all of the probe DNAs 1 in the microplate 2, thereby producing a biochip shown in Figure 4(e). In this manner, a biochip is conventionally produced by coating the binding agent for binding DNA to the glass on the entire surface of the plate before spotting the DNAs on the plate.

[0003]

Figure 5 is diagrams for illustrating the principle of hybridization using the biochip. Referring to Figure 5(a), sample DNA 11 labeled with a fluorescent substance 10 is hybridized in a hybridization solution with probe DNAs 1 that are spotted onto the glass plate 3 of the biochip via the binding agent 4. The hybridization solution is a mixture containing formaldehyde, SSC (NaCl, trisodium citrate), SDS (sodium dodecyl sulfate), EDTA (ethylenediamidete traacetic acid), distilled water and the like where the mixing ratio depends on the characteristics of the DNA used.

[0004]

When the sample DNA 11 is complementary to the probe DNAs 1 on the biochip, it binds to the DNA on the biochip and forms a duplex. On the other hand, the sample DNA 11 does not bind to probe DNAs that are not complementary thereto, and the sample DNA 11 labeled with the fluorescent substance 10 may bind to the binding agent 4 coating the glass plate 3, thereby remaining as garbage.

[0005]

Thereafter, as shown in Figure 5(b), the sample DNA 11 labeled with the fluorescent substance 10 remaining the glass plate 3 is washed away in water 12, whereby the sample DNA 11 that is not bound to the probe DNAs 1 is removed. Then, as shown in Figure 5(c), the fluorescent substance labeling the sample DNA bound to the probe DNA is excited with light from a lamp 14. The light emanated from the fluorescent substance is detected by an optical sensor 13 such as a CCD to detect the presence of hybridization.

[0006]

[Problems to be Solved by the Invention]

In an experiment using the biochip, the sample DNA is applied to the biochip to allow hybridization with the probe DNAs spotted on the biochip followed by detection of the probe DNA bound by the sample DNA. Following the hybridization and prior to the detection, the biochip is washed with water to remove the sample DNA that did not bind to the probe DNAs. However, since the entire surface of the plate is coated with the binding agent for binding the DNA to the glass, sample DNA that did not bind to the probe DNA adheres to the binding agent area of the glass plate where the probe DNAs are not located. The sample DNA 11 bound to the binding agent 4 cannot be removed from the glass plate 3 by washing with water. Such remainder sample DNA is detected as noise upon detection, rendering the detection sensitivity poor. In other words, some of the sample DNA that is not

specific to the probe DNA binds to and remains on the biochip via the binding agent 4 as garbage. When the fluorescent substance labeling the sample DNA is excited, the fluorescent light is detected as noise, whereby S/N (signal-to-noise) ratio is lowered.

[0007]

The present invention aims to solve this conventional problem, and provides a biochip in which sample DNA does not bind to area of the plate where the probes are not located. The present invention also provides a method for producing such biochip.

[0008]

[Means for Solving the Problem]

In order to accomplish the above object, the present invention provides a binding agent for binding probes and glass on a plate only where the probes are to be spotted. Since no binding agent is provided on the portions of the plate where the probes are not to be spotted, the sample DNA that does not bind to the probe upon hybridization can be removed away from the biochip by washing with water. Therefore, noise produced upon detection can be eliminated and thus the S/N ratio can be enhanced for high sensitivity.

[0009]

Thus, a biochip of the present invention comprises probes spotted on a plate at a plurality of positions by using a binding agent for binding the probes to the plate, wherein the binding agent is locally spotted at positions where the probes are spotted.

[0010]

The present invention is a method for producing a biochip by spotting probes on a plate by using a binding agent for binding the probes to the plate, the method comprising a step of spotting mixtures of respective probes and the binding agent on the plate.

[0011]

The present invention is a method for producing a biochip by spotting probes on a plate, the method comprising the steps of: spotting a binding agent for binding the probes to the plate at positions where the probes are to be spotted; and spotting the probes on the plate at positions where the binding agent is spotted.

The plate may be made of glass. Preferably, the probes are spotted with a pin with a recessed tip.

[0012]

The pin of the invention used for spotting the probes is provided with a recessed tip.

Moreover, a tip of a pin of the invention used for spotting a probe on a plate is provided with a cross-shaped groove.

[0013]

[Embodiments of the Invention]

Hereinafter, embodiments of the present invention will be described. Herein, DNA is used as a probe although the probe is not limited thereto, and RNA or protein may also be used as a probe. Although a glass plate is used in the examples, a nylon membrane or the like may also be used.

[0014]

Figure 1 is diagrams showing the principle of a first embodiment of the present invention. As shown in Figure 1(a), a microplate 2 contains various probe DNAs 1. As shown in Figure 1(b), a plate 3 to be incorporated into the biochip is made of glass. Referring to Figure 1(c), a binding agent 4 for binding DNA to glass is dispensed into each well of the microplate to be mixed therein with the probe DNAs 1. The binding agent 4 for DNAs and glass may be, for example, poly-1-lysine or carbodiimide.

[0015]

Then, as shown in Figure 1(d), each of the mixtures of the binding agent 4 and the probe DNAs 1 is suctioned by a pin 5 (or contacted and carried by the tip of the pin 5) and spotted onto the plate 3. This process is repeated for all of the probe DNAs 1 in the microplate 2, thereby producing a biochip 20 shown in Figure 1(e) in which the binding agent 4 is present only at the desired portions and is not present at portions where there is no probe.

[0016]

Figure 2 is diagrams for illustrating the principle of a second embodiment of the present invention. As shown in Figure 2(a), a microplate 2 contains various probe DNAs 1. As shown in Figure 2(b), a glass plate 3 is used as a plate of the biochip. As shown in Figure 2(c), a binding agent 4 is suctioned by, for example, a capillary tube 6 and applied on the glass plate 3 at positions where the probe DNAs are to be spotted. The binding agent 4 is spotted in advance on the glass plate 3 where the probe DNAs 1 are to spotted. Then, as shown in Figure 2(d), the probe DNAs 1 in the microplate 2 are suctioned with the pin 5 (or is contacted and carried by the tip of the pin 5) and repeatedly spotted onto the plate 3. Thus, as shown in Figure 2(e), a biochip 30 is produced in which the binding agent 4 is not provided on portions other than portions where the probe DNAs 1 are present.

[0017]

Figure 6 is schematic diagrams showing shapes of a tip (i.e., a portion with a concave shape where probes are to be contacted) of a pin 5 according to the invention. A pin 5b shown in Figure 6(b) has a concave tip with a cross-shaped groove. The concave shape of the tip of the pin allows the probe solution to be carried by surface tension by simply dipping the pin in the solution. The depth of the concave is optional. By using this pin, the amount of the DNA carried

can be increased by about 10 times or more the amount carried with a conventional pin with a flat tip. A pin 5c shown in Figure 6(c) has a flat tip with a cross-shaped groove. The amount of the DNA carried with this pin is also higher than that carried with the conventional flat tip.

[0018]

Figure 3 is diagrams for illustrating the principle of hybridization using the biochip of the invention. Referring to Figure 3(a), in a hybridization solution for hybridization, a sample DNA 11 labeled with a fluorescent substance 10 is placed together with the biochip 20 on which the probe DNAs 1 are spotted on the glass plate 3 via the binding agent 4. The hybridization solution contains formaldehide, SSC (NaCl, trisodium citrate), SDS (sodium dodecyl sulfate), EDTA (ethylenediamidetetraacetic acid) and distilled water where the mixing ratio differs depending on the characteristic of the DNA used.

[0019]

When the sample DNA 11 and any of the probe DNAs 1 on the biochip 20 are complementary to each other, both DNAs bind to each other and form a duplex. On the other hand, when both DNAs are not complementary to each other, they do not bind to each other and the sample DNAs 11 labeled with the fluorescent substance 10 remain on the glass place 3 as garbage. As shown in Figure 3(b), the sample DNA 11 labeled with the fluorescent substance 10 remaining on the glass plate 3 is washed away in water 12. Since the binding between the glass and the DNA is weak, the remaining garbage sample DNA 11 is removed away from the glass plate 3. Referring to Figure 3(c), the fluorescent substance labeling the bound sample DNA is excited with light from a lamp 14. The light emanated from the fluorescent substance is detected by an optical sensor 13 such as a CCD to detect the presence of hybridization.

Since there is no garbage sample DNA left on the biochip 20, the S/N ratio upon detection is enhanced.

[0020]

[Advantage of the Invention]

According to the present invention, a biochip can be produced in which a binding agent is locally spotted only where probes are to be spotted. Thus, the detection sensitivity upon reading the biochip can be enhanced.

[Brief description of the drawings]

[Figure 1] Diagrams for illustrating an example of a method for producing a biochip according to the present invention.

[Figure 2] Diagrams for illustrating another example of a method for producing a biochip according to the present invention.

[Figure 3] Diagrams for illustrating hybridization and detection using the biochip of the invention.

[Figure 4] Diagrams for illustrating a method for producing a conventional biochip.

[Figure 5] Diagrams for illustrating hybridization and detection using a conventional biochip.

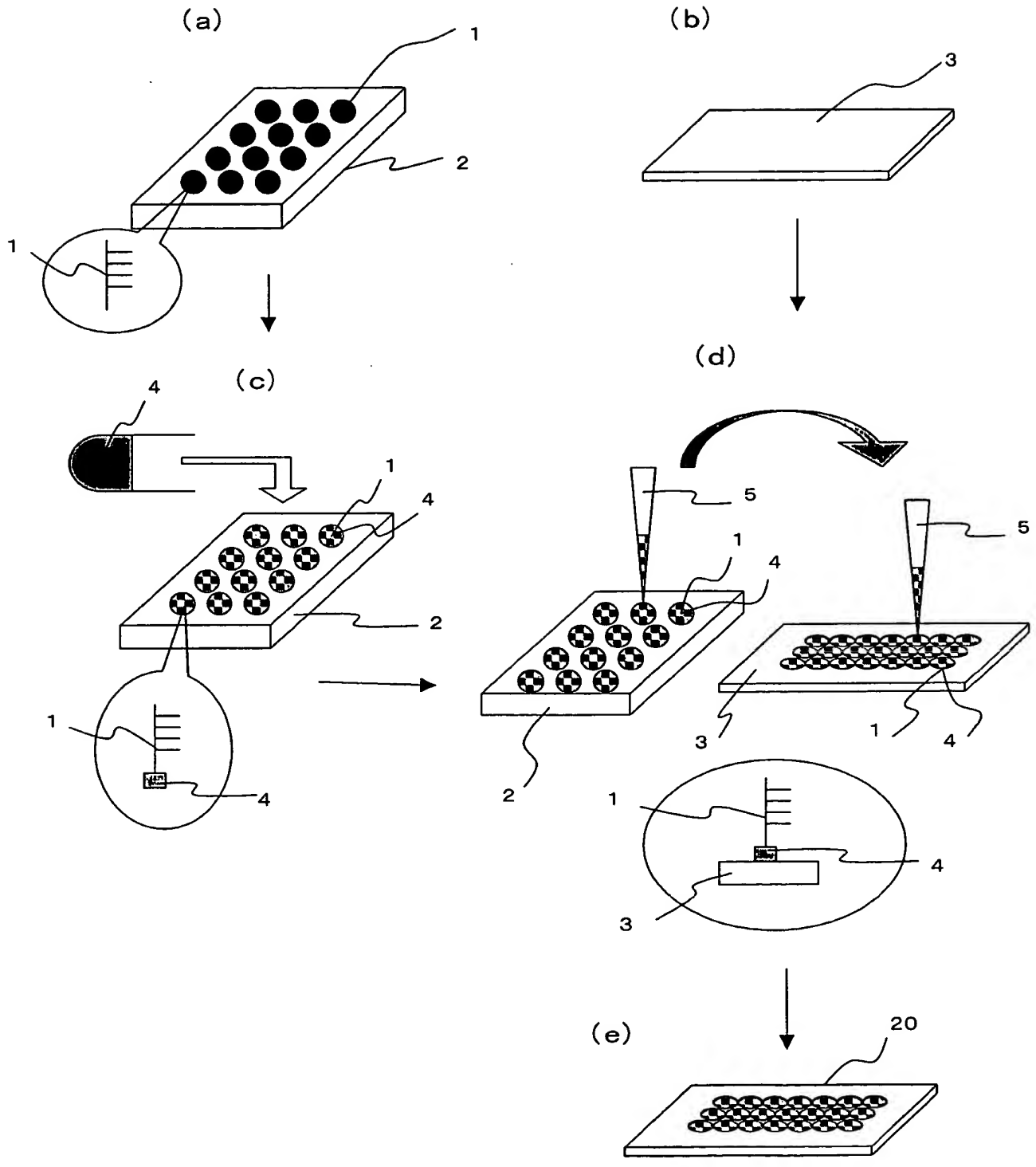
[Figure 6] Diagrams for illustrating a pin of the invention for spotting probes.

[Description of Reference Numerals]

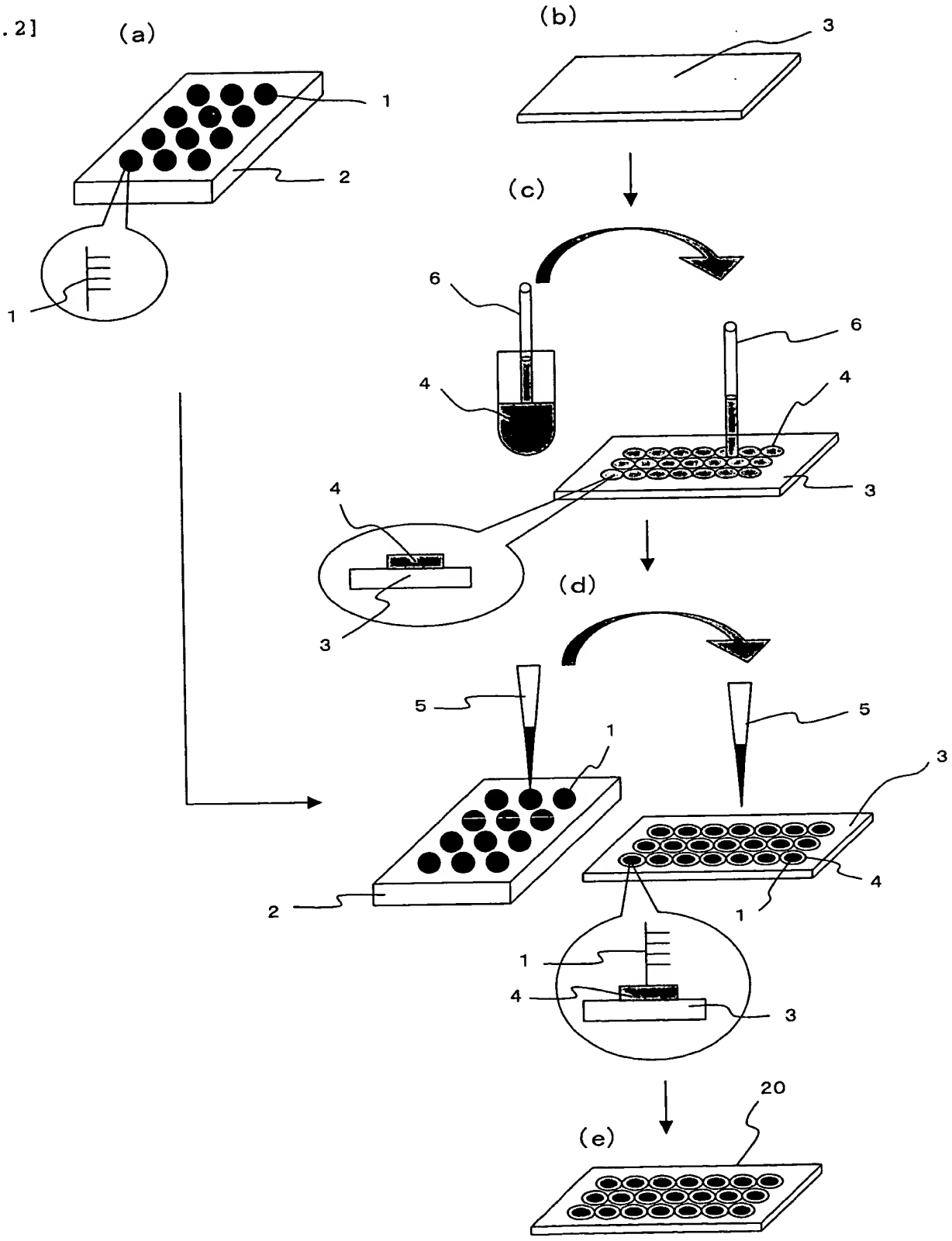
1...Probe DNAs, 2...microplate for storing probes, 3...glass plate, 4...binding agent, 5...pin, 6... capillary tube, 10...fluorescent substance, 11...sample DNAs, 12...water, 13...optical sensor, 14...lamp, 20...biochip

[Document Name] Drawing

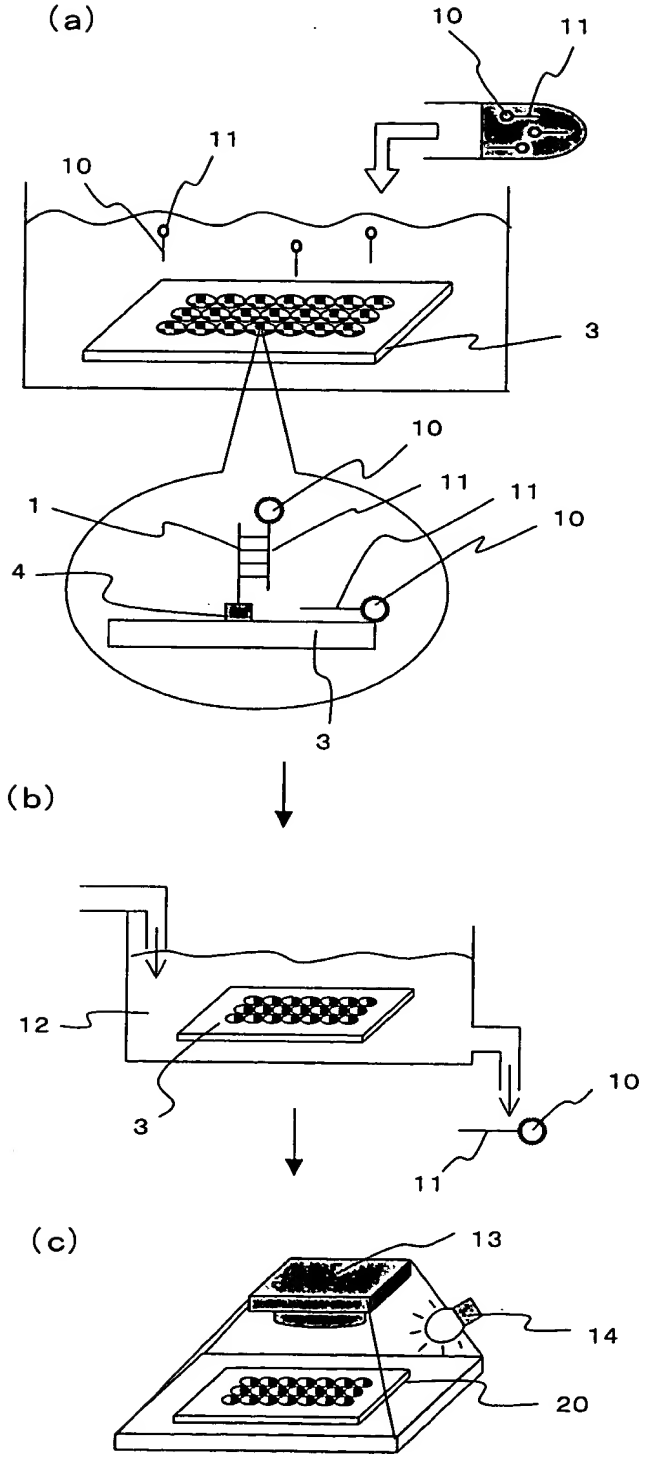
[Fig.1]



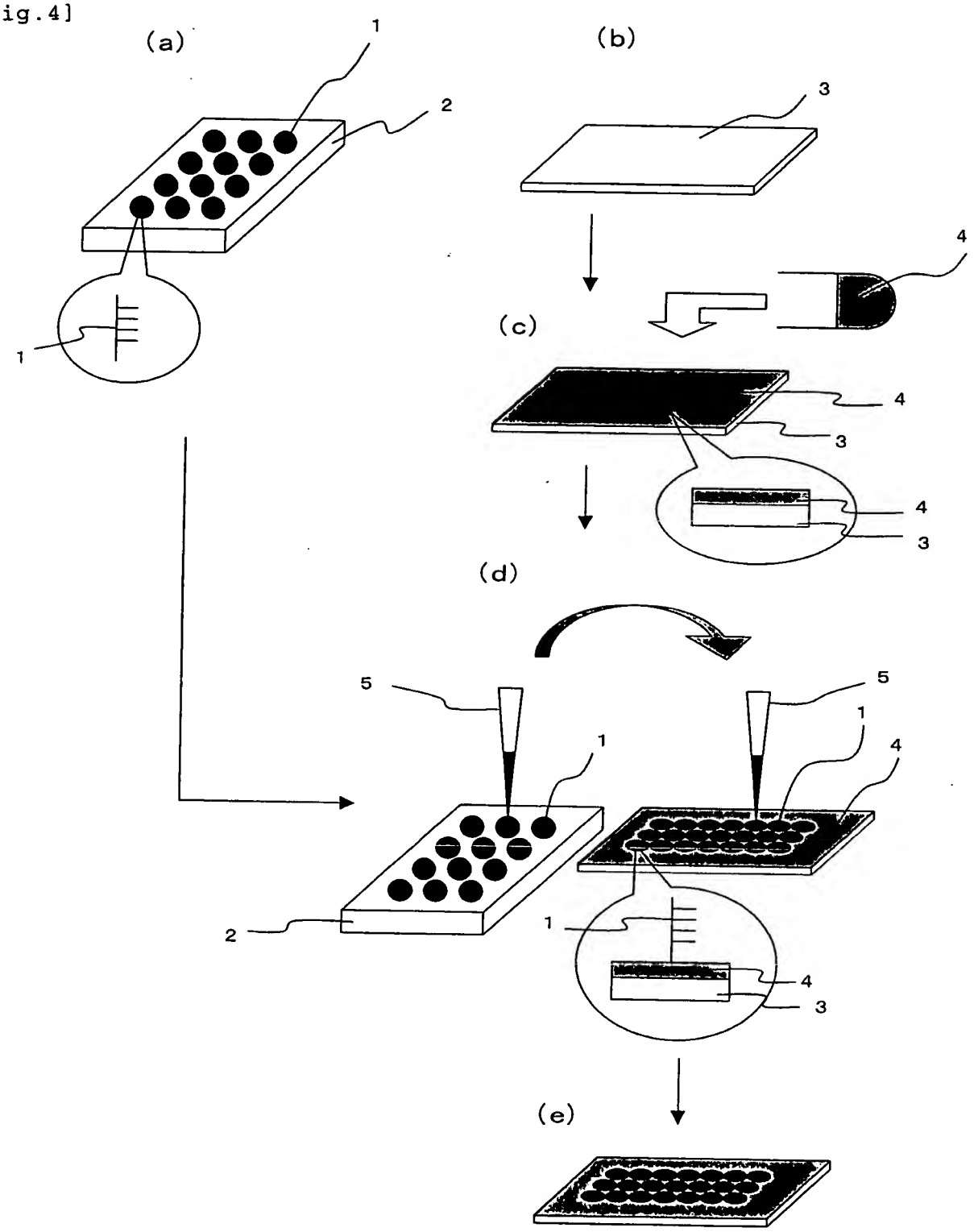
[Fig.2]



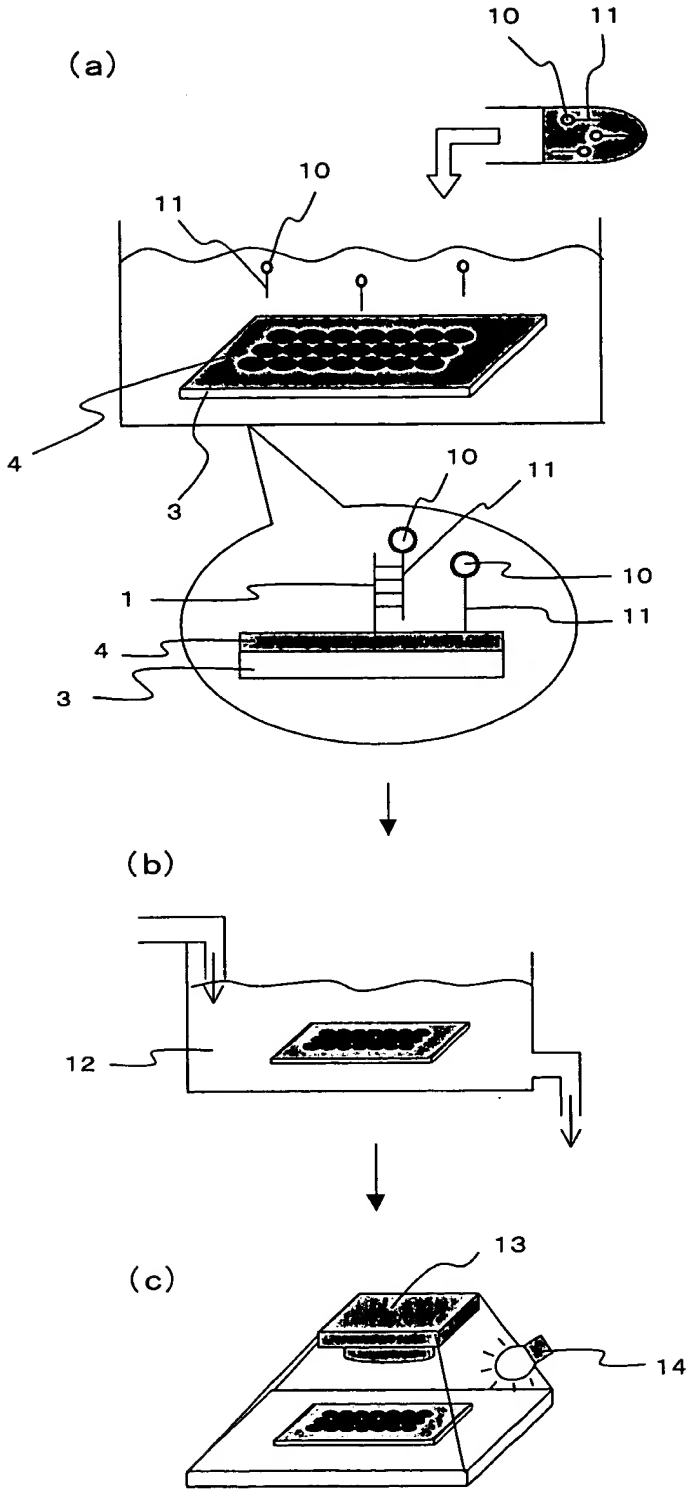
[Fig.3]



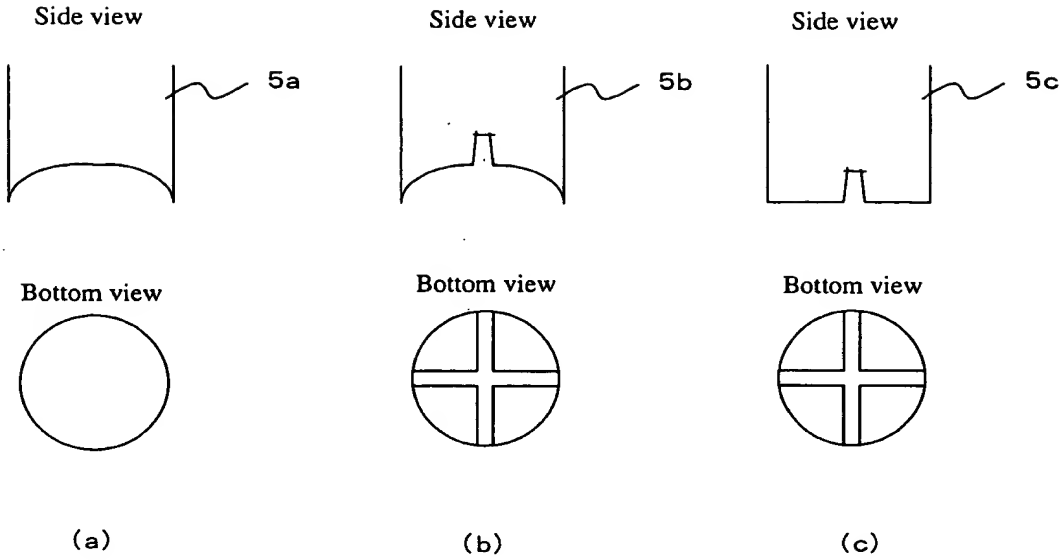
[Fig.4]



[Fig.5]



[Fig.6]


Side view
5a
Bottom view
(a)
Side view
5b
Bottom view
(b)
Side view
5c
Bottom view
(c)

[Document Name] Abstract

[Summary]

[Object] To provide a biochip on which sample DNAs are not on a portion of a plate of the biochip other than where probes are plotted.

[Means for Solving the Problem] Mixtures of probes 1 and a binding agent 4 are spotted on a plate 3. Alternatively, a binding agent for the probes and the plate is locally spotted on a plate where probes are to be spotted, and then probes are spotted on the plate where the binding agent is present. In this manner, a biochip 20 is produced on which a binding agent is not present on a portion of the biochip other than where probes are plotted

[Selected figure] Figure 1